



# **EDGEWOOD**

## **CHEMICAL BIOLOGICAL CENTER**

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

**ECBC-TN-018**

### **TESTS ON A LOW VOLUME MAIL SCREENING SYSTEM (LVMSS)**

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**April 2004**

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) XX-04-2004		2. REPORT TYPE Final		3. DATES COVERED (From - To) Aug 2003 - Sep 2003	
4. TITLE AND SUBTITLE Tests on a Low Volume Mail Screening System (LVMSS)				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Kesavan, Jana; Bottiger, Jerold; and Hottell, K. Aubrey*				5d. PROJECT NUMBER 622384/ACB2	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES) DIR, ECBC, ATTN: AMSRD-ECB-RT-TA, APG, MD 21010-5424				8. PERFORMING ORGANIZATION REPORT NUMBER ECBC-TN-018	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES *Student contractor assigned to Research and Technology Directorate, U.S. Army Edgewood Chemical Biological Center (ECBC).					
14. ABSTRACT A Low Volume Mail Screening System (LVMSS) was developed by Smiths Detection - Edgewood, Inc., to screen letters and packages delivered to a facility. The LVMSS was tested at the U.S. Army Edgewood Chemical Biological Center August 6-13, 2003. This was a quick test where a known amount of <i>Bacillus globigii</i> (BG) was placed in a letter (0.1 mg) or package (1 mg) before processing by the LVMSS. Each BG test was preceded by a control test without BG. Based on four letter and five package tests, the LVMSS correctly identified the tests with BG. The independent laboratory verification of the LVMSS's SpinCon® samples using the R.A.P.I.D. and Bio-Seeq® PCR methods showed positive for the tests with BG and negative for tests without BG. Reference filter samples indicated that there was no detectable BG exposure outside the LVMSS cabinet during BG letter and package processing. The nine tests conducted were not enough tests to estimate false positive and false negative statistics with good confidence.					
15. SUBJECT TERMS Low Volume Mail Screening System      Aerosol samplers      LVMSS Bacillus globigii      GeneXpert®      SpinCon®					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Sandra J. Johnson
U	U	U	UL	15	19b. TELEPHONE NUMBER (include area code) (410) 436-2914

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## **PREFACE**

The work described in this report was authorized under Project No. 622384/ACB2, Non Medical CB Defense. The work was started in August 2003 and completed in September 2003.

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### **Acknowledgment**

The authors acknowledge John Knapton's editorial assistance in preparing this technical note.

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## TESTS ON A LOW VOLUME MAIL SCREENING SYSTEM (LVMSS)

### 1. INTRODUCTION

A Low Volume Mail Screening System (LVMSS) was developed by Smiths Detection - Edgewood, Inc. (Edgewood, MD), to screen letters and packages delivered to a facility. Figure 1 shows a picture of the LVMSS. The LVMSS was tested at the U.S. Army Edgewood Chemical Biological Center (ECBC) from 6-13 August 2003. This was a quick test in which a known amount of *Bacillus globigii* (BG) was placed in either a letter or a package before LVMSS processing. Each BG test was preceded by a control test without BG. The tests did not represent a blind study, and there were insufficient test runs for a statistical analysis with good confidence of false positive and negative rates.

During the tests, employees from Smiths Detection operated the LVMSS, and ECBC employees observed the operations. The ECBC employees prepared the BG envelopes and packages for testing and were responsible for the collected samples before they were sent to independent laboratories for verification. Two reference filters located outside the LVMSS sampled the air during the test. Swipe samples of the LVMSS were also conducted to determine the system's contamination with BG. Reference filters, swipe samples, and part of each SpinCon<sup>®</sup> (Midwest Research Institute, Kansas City, MO) sample were archived and sent to the Microbiology/BSL-3 Facilities Team (J. Rogers) and the Special Programs Team (R. McClanahan) at ECBC for independent laboratory verification.

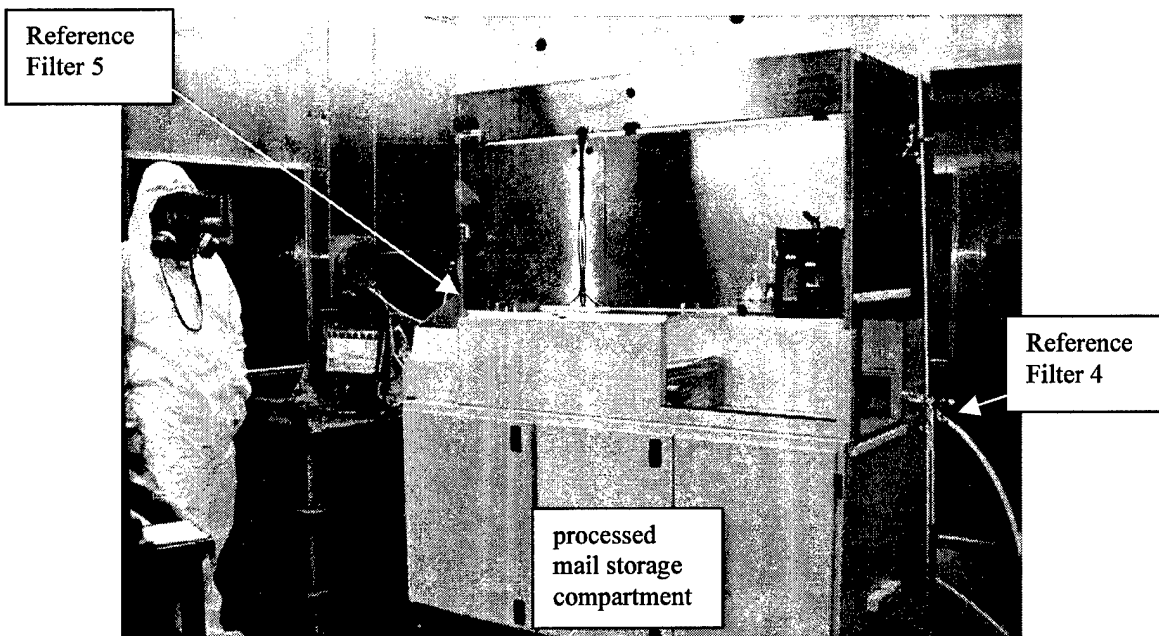


Figure 1. LVMSS.

## 2. METHOD

The appendix shows the test protocol. The LVMSS processes the letters and packages separately. BG letter tests were conducted with 0.1 mg of BG. Letters were stacked in the machine and pulled one by one by the machine. The edge of the letter was cut and squeezed to get the materials out of the letters in an enclosed area. Air from the enclosed area was sampled through a pre-separator into a SpinCon<sup>®</sup> air sampler. The analysis was conducted by the GeneXpert<sup>®</sup> (Cepheid, Sunnydale, CA). The processed mail was placed in a closed compartment while the analysis was being conducted. Each BG letter test was preceded by a control test without BG.

The package processing was done differently. Each package was opened, and a suction tube with a screen at the entrance was used to sample the inside of the package. The air samples were collected by the SpinCon<sup>®</sup>. The processed packages were placed in a closed compartment in the LVMSS (Figure 2) while the analysis was being performed. The BG package tests (each preceded by a control test) were conducted with 1 mg BG.

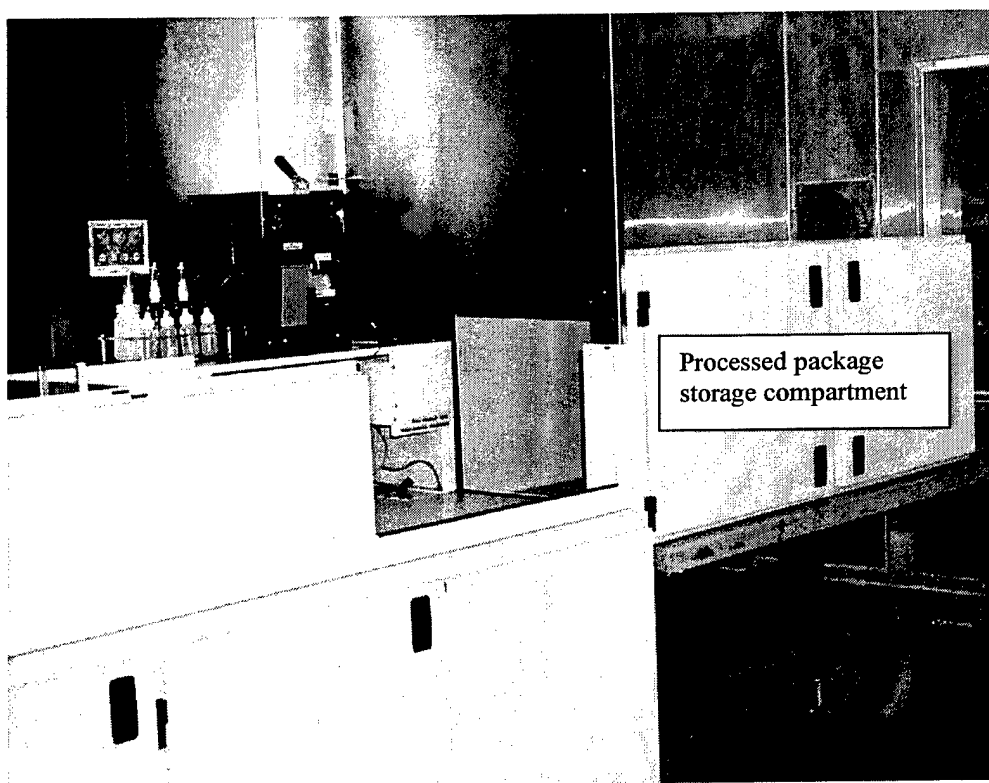


Figure 2. LVMSS with the Package Storage Compartment.

A liquid sample from the SpinCon<sup>®</sup> was collected at the end of all mail or package processing. One milliliter of 20% polyethylene glycol was added to the sample before a portion of the solution was manually placed in the GeneXpert<sup>®</sup> for analysis. After 35 min, the GeneXpert<sup>®</sup> system showed the results on the screen. The cycle threshold (CT) and end point numbers were recorded by ECBC personnel.

Reference Filter 4 (RF4) and Reference Filter 5 (RF5) were taken during each test to determine if there were any detectable BG exposure outside the LVMSS. The reference filters were placed on both sides of the machine as shown in Figure 1. The reference filters sampled the air while the SpinCon<sup>®</sup> in the LVMSS was sampling air, and the sampling time was recorded. The air flow rate of RF4 was 19.40 L/min and of RF5 was 17.64 L/min. Glass fiber filters were used as the sampling medium from which the BG was removed into liquid for independent laboratory verification using the Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D), Idaho Technology, Incorporated, Salt Lake City, UT, Polymerase Chain Reaction (PCR) method.

Five swipe samples were also taken before control tests to confirm that the LVMSS was free of BG and after the BG tests to determine the contamination location. The locations of swipes are shown in Figures 3-7. The swipe samples were analyzed by an independent laboratory using the R.A.P.I.D PCR method.

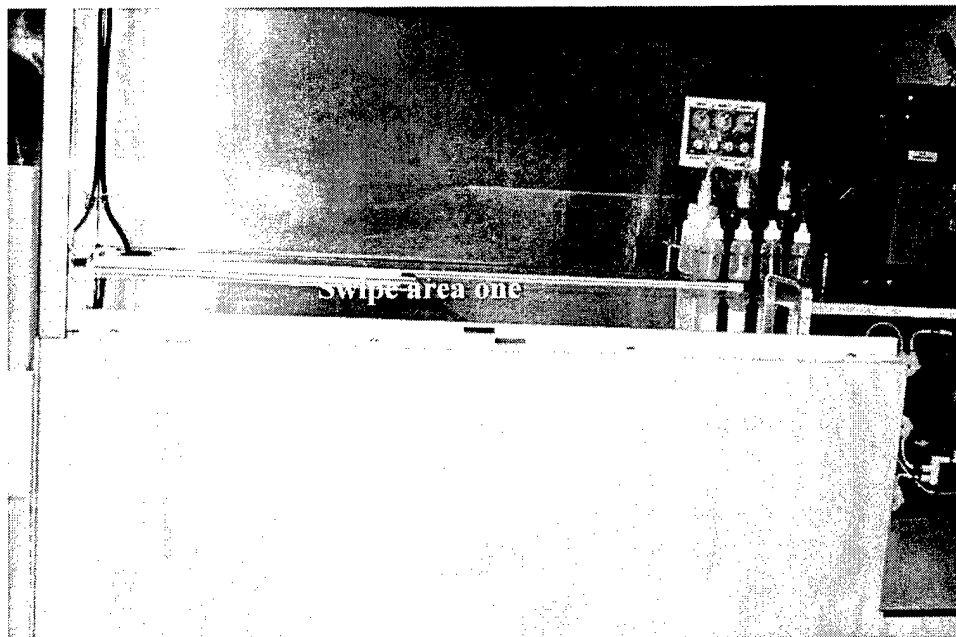


Figure 3. Swipe Area One on the LVMSS.

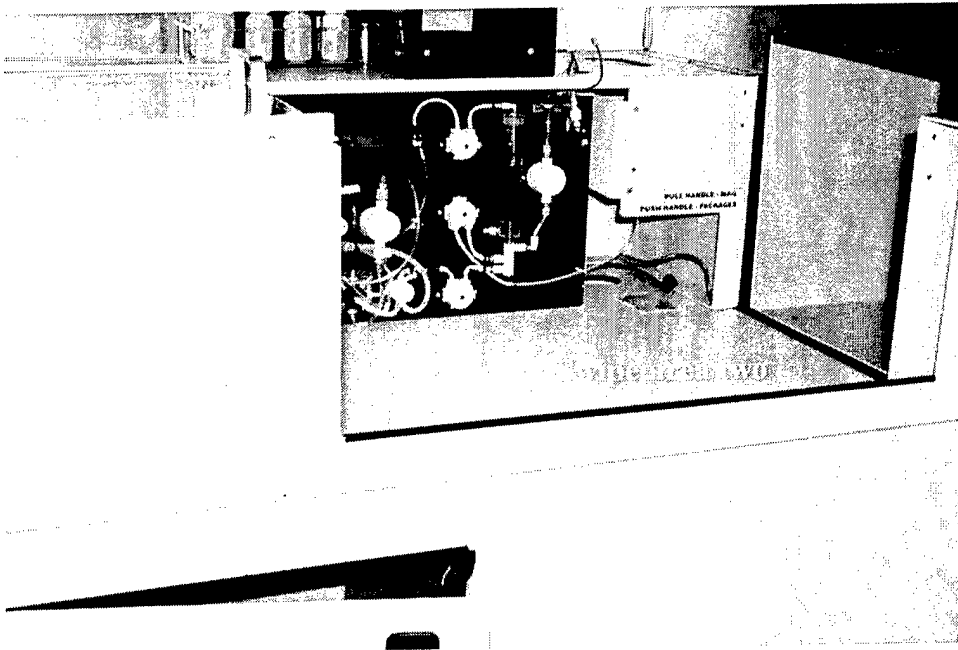


Figure 4. Swipe Area Two on the LVMSS.

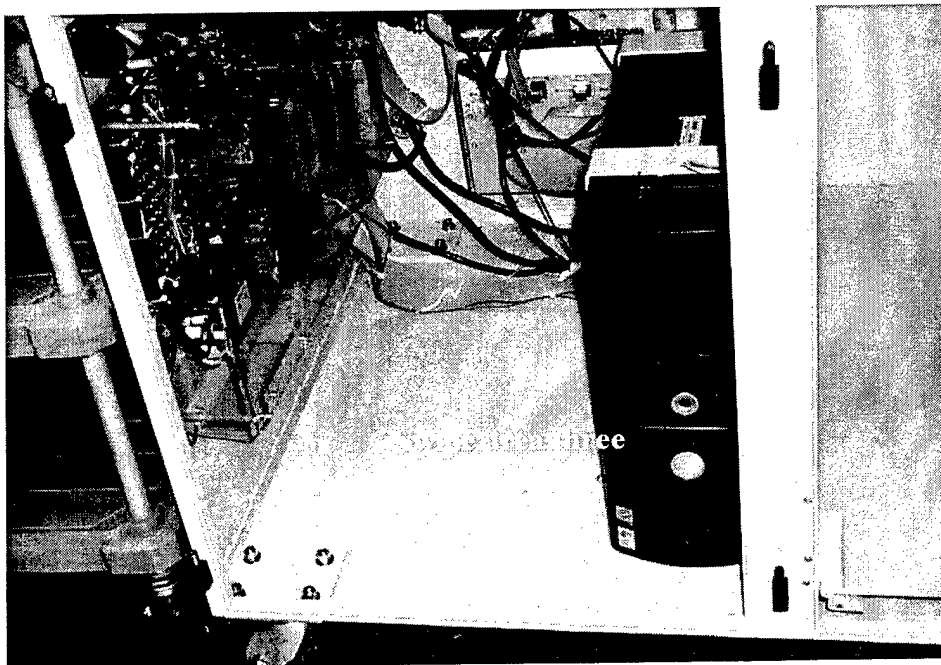


Figure 5. Swipe Area Three on the LVMSS.

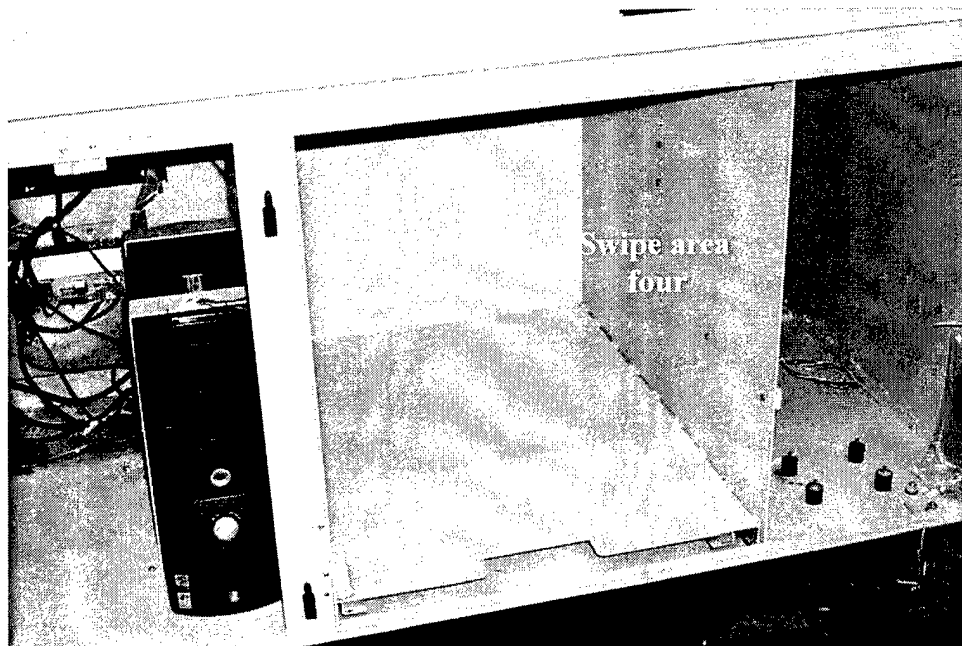


Figure 6. Swipe Area Four on the LVMSS.

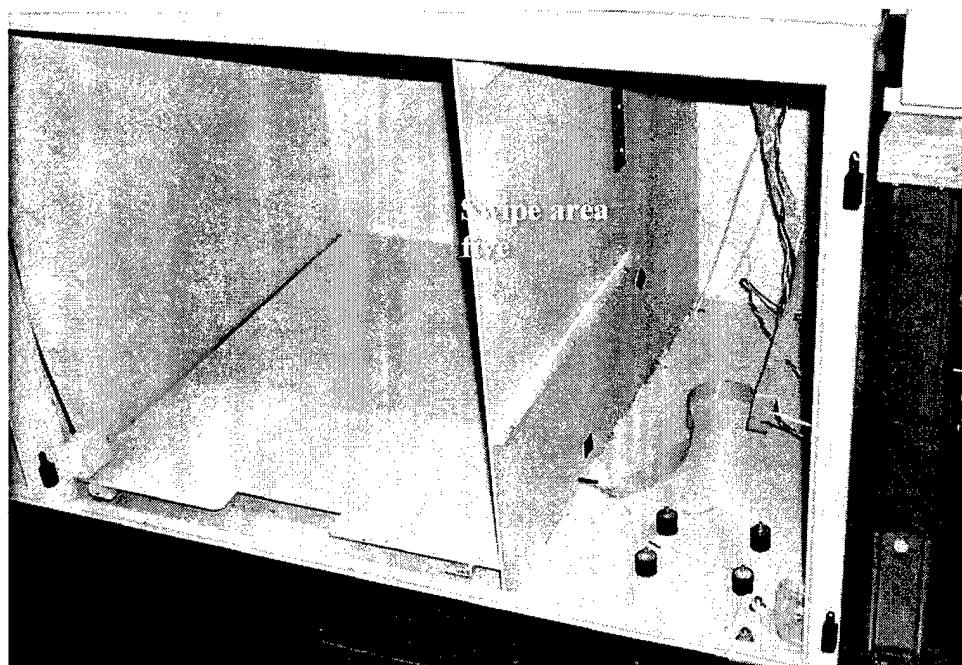


Figure 7. Swipe Area Five on the LVMSS.

Using the R.A.P.I.D. PCR method, the Microbiology/BSL-3 Facilities Team, ECBC, analyzed the collected SpinCon<sup>®</sup> samples, swipe samples, and reference filters. In addition, the Special Programs Team, ECBC, analyzed the SpinCon<sup>®</sup> samples using the Bio-Seeq<sup>®</sup> instrument (Smiths Detection). Data\* from J. Rogers and R. McClanahan describe the method of PCR used for analyzing these samples.

Before the tests began, the test chamber was decontaminated, and the LVMSS was tested to confirm that it was free of contaminant. After each BG test, the chamber and LVMSS were decontaminated. Following this decontamination to confirm that the LVMSS is fully decontaminated, the LVMSS's SpinCon<sup>®</sup> sampled clean air, and the liquid sample was analyzed using the GeneXpert<sup>®</sup>.

### 3. RESULTS

Four letter and five package tests were conducted. Test results are shown in the table herein. The length of time the RF sampled the air during the test is also provided. GeneXpert<sup>®</sup> response is shown in the table with CT and End Point values. Independent laboratory verification of the collected SpinCon<sup>®</sup> sample results using R.A.P.I.D. and Bio-Seeq<sup>®</sup> are shown in the last two columns.

The results show that the LVMSS clearly identified the BG exposure. However, the GeneXpert<sup>®</sup> in the LVMSS showed a positive result for a negative test (Run 7), while the R.A.P.I.D. and Bio-Seeq<sup>®</sup> showed negative results for the same SpinCon<sup>®</sup> sample. The observation that the GeneXpert<sup>®</sup> in the LVMSS returned a positive result, while the Bio-Seeq<sup>®</sup> and the R.A.P.I.D. returned negative results suggests that some residual contamination may have been located between the sample reservoir and the sample needle. In this scenario, the small amount (indicated by the high CT value of 40.92) of residual BG contamination would have been flushed into the sample compartment of the GeneXpert<sup>®</sup> cartridge. While this line is purged back into the sample reservoir (the archive of which was used for R.A.P.I.D. and Bio-Seeq<sup>®</sup> testing), if there was any BG that was not flushed to the cartridge, it would have been diluted to the point that it may not have been detectable to either the R.A.P.I.D. or the Bio-Seeq<sup>®</sup>. It should be noted that before this negative test (Run 7), the LVMSS was not run to confirm that it was fully decontaminated, as it was for all other tests (see the final step of the procedure in the appendix). It was the sponsor's decision to skip this step for this run because of the lateness of the hour and to check for successful decontamination of the LVMSS as a whole with the negative mail run.

The independent laboratory verification of the SpinCon<sup>®</sup> samples showed positive for the tests with BG and negative for samples without BG. Independent laboratory verification of the RFs and swipe samples showed negative for BG except for Run 2, swipe location 4, and Run 6, swipe location 2. Runs 2 and 6 were tests with BG, and it is likely that location 2 and 4 received BG exposure.

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\* Unpublished data sheets, 2003

Table. Results of Tests with the LVMSS.

Run No.	Test Type	Quantity of BG (mg)	RF Sample Time (min)	GeneXpert®					End Point TxR	PCR (R.A.P.I.D.) Results	PCR Bio-Seq® Results
				GeneXpert® Response	CT FAM	CT TxR	End Point FAM	End Point TxR			
1	Letter	0	7.5	Negative	?	32.91	?	278.12	Negative	Negative	Negative
2	Letter	0.1	23.6	<b>Positive</b>	32.3	34.74	229.21	138.21	<b>Positive</b>	<b>Positive</b>	<b>Positive</b>
3	Letter	0	7.3	Negative	-	32.43	-	303.07	Negative	Negative	Negative
4	Letter	0.1	28.6	<b>Positive</b>	34.68	35.2	181.48	159.38	<b>Positive</b>	<b>Positive</b>	<b>Positive</b>
5	Package with packing material	0	4.0	Negative	0	33.3	0.05	263.06	Negative	Negative	Negative
6	Package with packing material	1	8.1	<b>Positive</b>	29.48	34.29	266.59	114.4	<b>Positive</b>	<b>Positive</b>	<b>Positive</b>
7	Package with paper	0	4.3	<b>Positive</b>	40.92	34.2	62.97	267.91	Negative	Negative	Negative
8	Package with paper	0	4.3	Negative	0	33.52	-0.81	257.6	Negative	Negative	Negative
9	Package with paper	1	5.11	<b>Positive</b>	31.44	33.79	235.31	157.81	<b>Positive</b>	<b>Positive</b>	<b>Positive</b>

RF: Reference filter

CT: Cycle threshold

PCR: Polymerase chain reaction

CT TxR: Cycle threshold, Texas red dye

end point FAM: end point fluorescent dye

end point TxR: end point Texas red dye

R.A.P.I.D.: Ruggedized Advanced Pathogen

Identification Device

CT FAM: Cycle threshold, fluorescent dye

#### 4. CONCLUSIONS

Based on four letter (2 positive, 2 controls) and five package tests (2 positive, 3 controls), the Low Volume Mail Screening System (LVMSS) correctly identified the positive BG runs for letters containing 0.1 mg of BG and packages containing 1 mg of BG. The system correctly identified the control letter tests, and two of three control package tests. The other control package test (Run 7) is discussed below. The independent laboratory verification of the LVMSS's SpinCon<sup>®</sup> samples using the R.A.P.I.D. and Bio-Seeq<sup>®</sup> PCR methods showed positive for the tests with BG and negative for tests without BG.

The GeneXpert<sup>®</sup> system in the LVMSS identified the Run 7 sample as positive, while R.A.P.I.D. and Bio-Seeq<sup>®</sup> identified Run 7 samples as negative. Note that the standard check for LVMSS decontamination was not conducted before Run 7, and it is possible that there was a residual contamination between the sample reservoir and the sample needle in the LVMSS.

Reference filter samples verified that there was no detectable BG exposure outside the LVMSS during BG letter and package processing. There were only nine tests conducted, and these are not enough tests to estimate false positive and false negative statistics with good confidence.



## **APPENDIX**

### **TEST PROTOCOL**

#### **Tests without BG**

- Swipes of the LVMSS
- Chamber fans off
- Install reference filters
  - Reference filters turned on
  - Mail or package processing
- End mail or package processing and reference filters turned off
- Transfer SpinCon® Sample to GeneXpert®
- Start GeneXpert®
- Fans on
- Reference filters removed
- Collect SpinCon® samples for independent laboratory verification
- Mail or packages removed from chamber after negative tests

#### **Tests with BG**

- Fans off
- Install reference filters
  - Reference filters turned on
  - Mail or package processing
  - End mail or package processing and reference filters turned off
- Transfer SpinCon® Sample to GeneXpert®
- Start GeneXpert®
- Fans on
- Reference filters removed
- Collect SpinCon® samples for independent laboratory verification
- Swipe samples of LVMSS
- Mail or packages removed from chamber after positive tests
- LVMSS prep before chamber decon
- Chamber decon
- Decon LVMSS
- Run the SpinCon® and analyze using GeneXpert® before the next negative test to confirm that the system is clean.